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Anticonvulsant activity of the fractionated extract of *Crinum jagus* bulbs in experimental animals

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ABSTRACT

Objective: To investigate the anticonvulsant activity of the bulbs of *Crinum jagus* in experimental animals. **Methods:** The uprooted bulbs were air dried for a week and ground into creamy-paste. 200g of paste was macerated each in 2 litres of water, ethanol and petroleum ether and filtered after 48 h. The obtained filtrates were each evaporated at the appropriate temperature to solid residue. The residues were further fractionated with successive changes of petroleum ether, ethyl acetate and n-butanol into a pooled filtrate which was further evaporated to dry solid brown-paste. Phytochemistry was carried out based on Treas and Evans method of 1987. The acute toxicity study (LD50) was carried based on Lorke's 1983 method. Convulsion was induced using maximum electric shock (MEST), pentylenetetrazole(PTZ), strychnine and Picrotoxin in the appropriate animal models. Seizures onset time and death time were used as successful induction of convulsion while prolongations of these features were taken as anticonvulsant activity. Results where possible, were statistically analyzed using SPSS-16.0 version. **Results:** The LD_{50} was got at 1118.003mg/kg (IP) in mice using Lorke's 1983 method. Fractionated extract of *Crinum jagus* exhibited dose dependent antiseizure against MEST induced seizure ($P<0.001$) and comparable to that of phenytoin, a standard anti generalized tonic-clonic seizure. There were also observable antiseizure activity of the fractionated extracts against PTZ, strychnine and Picrotoxin induced seizure and comparable to their standard corresponding antiseizures. **Conclusions:** We conclude that the bulbs of *Crinum jagus* possess proven broad spectrum antiseizure and perhaps antiepileptogenic activity thus justifies its use in traditional medicine. Clinical trial in man is recommended.

1. Introduction

Seizure is coined from the Latin word Sacire "To take possession of". Seizure (Convulsion) is therefore a paroxysmal event due to abnormal, excessive hyper-synchronous discharges from aggregates of central (Cerebral) neurons[1].

Although, a variety of factors influence the incidence and prevalence of seizures, approximately, 5%-10% of the population will have at least one seizure during their life time with the highest incidence occurring during early childhood and late adulthood and because seizure is common, this clinical condition is encountered frequently during medical practice in a variety of settings[1]. Seizures could occur in a variety of clinical settings, including febrile convulsion that is common in children, head injury, enclampsia in pregnancy, frank epilepsy, septicaemia, tetanus, meningitis, stroke, metabolic disorders and could be of different kinds[2]. Seizure in epilepsy is unprovoked, intractable and reoccurring unlike seizures in other settings that could be secondary[1,3].

Most epileptic patients do not only suffer from stigmatization, they usually suffer from depression, muscular spasm, strange sensations, abnormal behavioural changes, convulsions, loss of consciousness and are highly prone to suicide and sudden, unexpected death[4]. Epilepsy affects natural intelligence among a studied group of Nigerian epileptics[5]. Recurrent excitatory activity or increased latency period of convulsion results in neurosis and dementia, as glutamate damages the brain cells[6].

Current drug therapy tends to target certain strategies in the pathogenesis of seizure. The dentate gyrus (DG) is a gateway that regulates seizure activity in the hippocampus. Lamotrigine when used as an anticonvulsant, blocks both

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AMPA and NMDA receptors of glutamate[7]. During seizures, electrical impulses are distributed from one neuron in the brain to another through ion channels. Major ion channels include Sodium and Calcium. Therapeutic drugs in current use are therefore employed to block these ions thus correct the spread of seizure. Notable examples are phenytoin and carbamazepine that block Sodium ions and are thus used for grandmal seizures. Ethosuximide and valproic acid that block calcium ions in T-channel, are used for Petitmal seizures. A counter action of gamma amino butyric acid (GABA) which is inhibitory to the excitatory effect of glutamate/aspartate is employed in therapy. This includes the use of benzodiazepines or barbiturates as GABA-enhancers, gabatin, pregabalin and gabapentin as GABA analogues and vigabatrin or tiagabin as GABA hydrolysis inhibitors. Direct blockade of glutamate receptors is also employed in therapy. Lamotrigine acts in this manner[7].

These drugs/strategies however bring about only symptomatic relief as they do not control epileptogenesis, the process by which the brain develops epilepsy[8] [9].

Current research into antiseizure therapy is looking into the possibility of synthesizing drugs that can block the gliotransmission of ATP (adenosine tri-phosphate). This transmission in astrocyte-glia cells leads to astrocyte-calcium wave generation. Blockade of ATP signaling will not only decrease the frequency of epilepsy, it will make more adenosine available through further hydrolysis of ATP[8]. The list of anti seizures has even tripled in the 21 first century but, epilepsy remains a big clinical burden as no definite cure/control without devastating adverse effects appears to be in sight[9].

More than one third of patients with epilepsy have inadequate control of seizures with drug therapy, as most drugs in current use are largely palliative and there currently exist no drug that has eliminated or prevented reoccurrence of epilepsy (Antiepileptogenic) [2].

An intriguing possibility is to control acquired epilepsy by preventing epileptogenesis, a process by which the brain becomes epileptic. A number of antiepiletics have been evaluated in clinical trials to test whether they prevent epileptogenesis in humans, but to date no drug has been shown to be effective in such trials. Thus, there is a pressing need for drugs that are truly antiepileptogenic to either prevent epilepsy or alter its natural course[9]. More than 80% of the world's population use or has at one time or the other used, or resorted to herbal remedy for treatment of ailments[10]. Medicinal plants have proven to be very good therapeutic agents just like the orthodox drugs but, unlike orthodox drugs, are said to exhibit only minimal or no side/adverse effects[11,12]. The aqueous extract of stem back of Diospyros fischeri Gurke (Ebenaceae) had no effect on Picrotoxin induced convulsion in mice, the ethanolic extract showed a dose-dependent suppression of convulsion[13]. Unlike other studies, the hydro-alcoholic extracts from Erythrina velutina and Erythrina mulungu have only anticonvulsant effects only on Strychnine-induced seizure model, suggesting their possible action on glycine system and a potentiation of pentobarbital sleeping time, suggesting depressant action on the central nervous system[14].

Crinum is the largest tropical genus of the amaryllidaceae/lilaceae family[15]. *Crinum jagus* is a medicinal plant used in traditional medicine either singly or in a combination with Chromoleana odorata and Emilia prateramisa (both of Asteraceae family) in treatment of all forms of convulsive state. *Crinum jagus (C.jagus)* is variable specie that occurs in tropical Africa (Figure 1). Its leaves may be broad in some forms, whereas they are narrower or parallel in other forms. The plant may be found in swampy conditions, seasonal wetlands or in grassland (Savanna). Some plants are remarkably fragrant, flowered, vanilla scented while others may have little or no scent/odor. Buds are enclosed in several sheathing bracts (Figure 1.1). The bulbs are ground, macerated in hot palm-oil and used in treatment of all types of seizures[16]. Morpholine, Hamayne and Lycorine have earlier been isolated as active alkaloids of *C. jagus*. Lycorine has been shown to possess anti-tumor activity and shall be available in commercial quantity[17]. Earlier work on the crude extract of *Crinum jagus* revealed anti mitotic activity[18]. In India, *Crinum jagus* is used in treatment of snake bites[19]. *Crinum jagus* bulb has also recently been shown to possess anti-asthmatic activity[20]. In another recent work antioxidant and antihaemorrhagic activities of *C.jagus* were documented[15]. The bulbs of *Crinum jagus* is also used in treatment of chronic cough, malaria, sores and possess antibacterial activity[21].

Figure 1. *Crinum jagus.*

2. Materials and methods

2.1. Total number of animals used for the study

Consisted of 53 Wister Albino Mice, 20 adult Wister Albino Rats and 20 day-old Chicks. Drugs consisted of convulsant models and standard anticonvulsants.

2.2. Plant collection and taxonomy

The plant was collected on the 15th of January, 2008 from Igbodo in Ika N/East Local Government Area of Delta State, Nigeria. The plant was identified as *Crinum jagus* a member of the family amaryllidaceae/lilaceae by Mr A Ozioko, of the Department of Botany, (Herbarium section), University of Nigeria, Nsukka[11]. A voucher specimen of the plant has been kept in the University's herbarium for future reference.

2.3. Plant extraction

Extraction was divided into two stages. The first stage involved crude extraction in polar (water), semi polar (ethanol) and non polar (petroleum ether) solvents. The second stage involved further extraction (fractionation) of the pooled crude

extract gotten from the first stage[16].

2.4. Phytochemistry

Method of Phytochemistry was based on the method of Trease and Evans (1987) and as applied by Azikiwe *et al* [22]. Phytochemistry was carried out to test for presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, reducing sugars, fats/oils, resins, proteins, acidic substances and carbohydrates.

2.5. Acute toxicity

Lorke's 1983 involved the use of 13 animals and divided into two stages. At the first phase, a total of 9 male mice of average weight of 20.5g were used[11,23]. The animals were divided into 3 groups (A-C) of 3 animals per group. The fractionated extracts of *Crinum jagus* re-dissolved in water and administered intraperitoneally to the mice at doses of 10mg/kg, 100mg/kg and 1000mg/kg. All animals had unrestricted access to water and animal feed and were then observed in their cages for 24h. The animals were observed for possible signs of toxicity (anorexia, drowsiness, apnoea, immobility, twitches and irritation) and, or death. All dead animals were immediately removed from the cage as soon as possible once death was observed, counted and recorded. The second stage followed the end of the first stage and 4 new mice were selected and placed 1per cage. They were separately given 2500mg/kg, 5000mg/kg, 7500mg/kg and 10000mg/kg and were further observed for the next 24 h. LD_{50} was then calculated as the geometric mean of the lowest dose that killed an animal (2500) and the highest dose that did not kill any animal (1000). Geometric mean was calculated as the average of the square roots of the lowest dose that killed an animal and the highest dose that did not kill any animal. With the LD_{50} obtained, the presumable effective doses were taken thus one-fifth of LD_{50} as high dose (HD) and one-tenth as low dose.

2.6. Convulsion induction models

Convulsion was induced in different animal models using maximum electric shock(MEST), pentylenetetrazole(PTZ), strychnine(Sty) and picrotoxin(Picro). While maximum electric shock was used in 25 day old chicks, PTZ was used in 25 adult rats and Sty and Picro were used each in 25 adult mice. The methods employed standard methods[13,14,16]. Warm red palmoil(2mL/kg) was used as transport vehicle control to account for its use by traditional healers. Pure drinking (2mL/kg) was used as negative control.

3. Results

3.1. Extraction

The aqueous filtrate had a pH of 5 and boiled at 108℃. The starting paste of 200g of the bulbs of *Crinum jagus* for each of Petroleum-Ether (PE), Ethanol (ET) and Water (WT) as extracting solvents, resulted in 170.2g, 124.5g and 54.02g crude extraction for PE, ET and WT respectively. Percentage yield was got as 85% for PE, 62% for ET and 27.01% for WT (Table 1).

Table 1
Solvent extraction of *Crinum jagus* bulbs.

	Petroleum ethar	Ethanol	Water
Weight of *C.jagus*	200g	200g	200g
Volume of olvent	2L	2L	2L
Volume of filtrate	1.96L	1.96L	1.90L
Boiling point	–	–	108 ℃
Ph	6.6	6.8	7.0
Extract	170.2g	124.5g	54.02g
% Extract yield	85.0	62.0	27.01

3.2. Fractionation

Fractionation started with a sum weight of 348.72 g and came out with a total weight of 342.5 g, percentage fractionation of 98.22%. The fractionated extract had brownish-slimy appearance. It had a sweet smell but, slightly choking on deep inhalation. The fractionated extract dissolved in water to form a brownish emulsion-like solution with a pH of 6 (Table 2).

Table 2
Fractionation of *Crinum jagus* bulbs.

	Petroleum ether	Ethyl acetate	*n*-Butanol
Total volume	100mL	100mL	100mL
Extract	→	→ 348.72g←	←
Fractions	97.2mL	97.9mL	99.8mL
Ph	6.7	6.65	6.3
Pooled F/extract	→	→ 342.5g←	←
Loss fraction	→	→ 6.22g←	←
% Loss fraction	→	→ 1.78%←	←
Dissolution/ Ph	→	→ 6.0←	←

Arrow indicates compounding or pooling effects.

3.3. Phytochemistry

The phytochemistry revealed that Alkaloids and flavonoids were abundantly present. Saponins, Tannins, Fats/Oils were significantly present. Reducing sugar, Carbohydrate, Proteins and glycosides were moderately present. Acidic compound was present while Steroids, Terpernoids and Resins were absent (Table 2).

Table 2
Phytochemistry results.

Tested substances	Results
Carbohydrate	+ +
Reducing sugar	+ +
Alkaloids	+ + ++
Saponins	+ + +
Flavonoids	+ + ++
Steroids and terpernoids	–
Proteins	+ +
Tannins	+ + +
Glycosides	+ +
Resins	–
Acidic compounds	+
Fats/Oils	+++

– = not present; + = present; ++= moderately present; +++= significantly present; ++++ = abundantly present.

3.4. Acute toxity study

At stage one, no animals died (Table 3) but doses of 2500 to 10,000mg/kg in stage two however, killed the animals (Table 3), thus LD_{50} was taken as the geometry mean. Thus: 2500 (lowest dose that killed an animal) X 1000(Highest dose that killed no animal) =2,500,000 divide by 2 =1,250,000. Take square root= 1,118.033mg/kg. LD_{50} for fractionated extract of the bulbs of *Crinum jagus* is 1118.033mg/kg in Mice(IP).(Using Lorke's method) (Tables 3 and 4).

Table 3
Stage one LD_{50} study.

Doses of extract	NO of animals	Death
10mg/kg	3	0
100mg/kg	3	0
100mg/kg	3	0

Table 4
Stage two LD_{50} study.

Doses of extract	NO of animals	Death
2500mg/kg	1	1
5000mg/kg	1	1
7500mg/kg	1	1
10,000mg/kg	1	1

3.5. Anticonvulsant study

3.5.1. Anticonvulsant activity in day-old chicks

Fractionated extract of *Crinum jegus* exhibited a dose related anti seizure activity agaist MEST induced seizure (P<0.001). This was comparable to that of phenytoin (P<0.01) which though had longer seizure onset time but, a shorter death time than fractionated extract.

Crinum jagus like most medicinal plants formulation by traditional medicine healers is macerated in palm oil. The present study could not demonstrate any significant antiseizure activity of palm oil used (Table 4).

Table 4
Average seizure onset time and death time.

Doses	Seizure onset time (min)	Death time (min)
Diazepam-5mg/kg	45.0±0.31*	50.6±0.4*
F/Extract-120mg/kg	34.4±0.2*	42.0±0.5*
F/Extract-240mg/kg	40.4±0.2*	50.4±0.2*
Water-2mL/kg	6.4±0.2	8.6±0.4
Palm oil-2mL/kg	7.6±0.4	9.6±0.4

* indicates results with significant difference from negative control.

3.5.2.Anticonvulsant activity against pentylenetetrazole induced Seizure

In the present study seizure was achieved at 90mg/kg from our earlier pilot study unlike that of Oliveria *et al*[24], that achieved seizure at 80mg/kg.

With PTZ induced, there was statistically insignificant antiseizure activity of red palm oil. Fractionated extract also exhibited actiseizure activity in dose dependent fashion (P<0.001).

High dose of fractionated extract had anti PTZ induced seizure activity statistically equal to that of Valproic acid, a standard anti PTZ induced seizure (Table 5). The animals under Valproic acid and F/Extract exhibited intermittent seizures and showed increase in seizure onset time but, later died at their final average time of 179.8min for valproic acid, 110.6min for low dose and 183.8min high dose of fractionated extract.

Table 5
Average seizure onset time and death time.

Doses	Seizure onset time (min)	Death time (min)
Phenytoin-5mg/kg	49.0±0.7*	60.4±0.5*
F/Extract-120mg/kg	20.4±0.9*	31.2±0.4*
F/Extract-240mg/kg	44.2±0.2*	60.6±0.4*
Water-2mL/kg	6.3±0.2	7.8±0.2
Palm oil-2mL/kg	6.8±0.2	8.4±0.4

* indicates results with significant seizure activity compared with control.

3.5.3. Anticonvulsant against seizure induced by Strychynine

Seizure onset for fractionated extracts stood at an everage 34.2 for low dose and 40.8 for high dose while that of diazepam, a standard anticonvulsant was 45.2min. The death times were 50.6 for diazepam, 41.8 for low dose F/extract and 51.2 for high dose F/extract. The high dose of F/extract showed a higher resistance to death thus a superior antiseizure compared to diazepam (P<0.03) (Table 6).

Table 6
Average seizure onset time and death time.

Doses	Seizure onset time(min)	Death time (min)
Valproic acid-200mg/	90.4±0.2*	179.8±0.4*
F/Extract-120mg/kg	91.0±0.3*	110.6±0.4*
F/Extract-240mg/kg	120.2±0.2*	183.8±2.3*
Water-2mL/kg	8.0±0.3	9.0±0.3
Palm oil-2mL/kg	10.4±0.2	15.0±0.3

* indicates results with significant ant seizure activity compared with control.

3.5.4. Anticonvulsant against seizure induced by Picrotoxin

The results of Strychynine and Picrotoxin induced seizures were similar but, unlike that of strychnine, that of Picrotoxin had no difference in death time of high dose fractionated extract (50.4min) compared with diazepam (50.6min). The results of diazepam and fractionated extracts were again similar and higher than that of control (Table 7).

Table 7
Average seizure onset time and death time.

Doses-mg/kg	Seizure onset time (min)	Death time (min)
Diazepam-5mg/kg	45.2±0.4*	50.6±0.2*
F/Extract-120mg/kg	34.2±0.2*	41.8±0.6*
F/Extract-240mg/kg	40.8±0.5*	51.2±0.8*
Water-2mL/kg	6.2±0.2	8.4±0.2
Palm oil-2mL/kg	7.4±0.2	8.6±0.2

* indicates results with significant ant seizure activity compared with control.

4. Discussion

The present study found that both the crude and fractionated extract of *C. jugus* were oily in appearance and contained mainly non polar compounds which were possibly responsible for its observed pharmacological activities. The fractionated extract was found to be very rich in alkaloids, flavonoids, resins and acidic compounds, safe and had a broad spectrum of anti-seizure activity.

The fact that the fractionated yield was highest with N-buthanol, a non polar solvent and that the fractionated extract was oily in appearance indicated the active constituents of the fractionated extract were very likely non-polar. These findings are in agreement with previous findings of Edema and Okaniemen[16].

Previous work on *C. jagus* identified three principal constituents namely: morpholine, lycorine and hamayne as well as calcium salts as other flavonoids components[16]. Other than the calcium salts, these compounds are essentially alkaloids and flavonoids[17-20]. Phytochemistry of the fractionated extract demonstrated the rich presence of alkaloids, flavonoids, tannins and acidic compounds. It had been expected that fractionated extract would be alkaline judging from the rich presence of alkaloids but the contrary was true. Subramoniam had reported that the extraction of medicinal plants increases their acidity and toxicity. Acidity of the fractionated extract may be related to the presence of calcium and oxalate salts or the high carbon atoms of the polyphenolic compounds (flavonoids) as well as the presence of acidic compounds.

As stated earlier in the methods, inhibition of MEST-induced seizures signifies anti-generalized tonic-clonic, myoclonic and atonic (grandmal) seizure while inhibition of PTZ-induced seizures implies inhibition of generalized non-convulsive (absence) seizures. The fractionated extract showed strong activity against MEST-induced seizures, moderate activity against strychnine- and picrotoxin-induced seizures and mild activity against PTZ-induced seizures. The seizure inhibitory activity of the fractionated extract was dose-dependent and comparable to that of standard anticonvulsants. Alkaloids and flavonoids have been shown to possess anticonvulsant activity. Their mechanism of anti-seizure activity may be due to their antioxidant activity or through some vague means. These substances are richly found in *C. jagus* and its antiseizure activity against MEST-induced seizures may stem from their presence.

Lycorine has been shown to possess anti-tumor but not antiseizure activity. Morpholine from yet to be published work in our Lab has no statistically significant activity against MEST, Strychnine- and picrotoxin-induced seizures. The broad spectrum anti-seizure activity of the fractionated extract may therefore be attributable to hamayne the third of the major constituents of *C.jagus* (lycorine and morpholine being the others) or a synergistic effect of the components of *C. jagus*. It is also possible that there exist other active constituents of *C. jagus* that are yet to be identified.

We conclude that the bulbs of *Crinum jagus* possess proven broad spectrum antiseizure and perhaps antiepileptogenic activity thus justifies its use in traditional medicine. Clinical trial in man is recommended.

Conflict of interest statement

We declare that we have no conflict of interest.

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